

THE OHIO JOURNAL OF SCIENCE

VOL. XXVII

NOVEMBER, 1927

No. 6

STUDIES ON THE PHYSICAL PROPERTIES OF LEAVES AND LEAF SAPS.*†

BERNARD S. MEYER,

Department of Botany, Ohio State University.

The ability of the leaves of certain species of plants to endure without injury temperatures below freezing for protracted periods of time is known to bear a close relation to the physico-chemical properties of the leaf cells. Recent advances in our knowledge of drought resistance seem to show that this property also may have its basis in the physiology of the leaf cells, rather than in anatomical peculiarities of the leaves. Studies of the physical properties of the cell and of the protoplasm may be expected to advance our knowledge of these phases of plant physiology. The relative drought resistance of different species of plants is an important factor in ecological distribution. The survival of the leaves of certain species of herbs, shrubs, and trees during the winter months can only be explained when the basis for frost resistance in the leaves of these species is understood. From the practical standpoint, knowledge of those physical properties which are correlated with frost and drought resistance will permit predictions regarding the suitability of various varieties of agricultural species for cultivation in localities subject to climatic extremes. If no physiological measures of relative frost or drought resistance be available, the alternative method of field tests must be used, comparatively costly in both time and money.

* Contributions from the Department of Botany, the Ohio State University, No. 203.

† Dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Ohio State University.

The fundamental importance of the water relations of plants in determining their distribution has long been recognized. The universally accepted ecological classification of plants by Warming (53) into hydrophytes, mesophytes, and xerophytes recognizes this basis. Transeau (52), and later Livingston and Shreve (38, p. 518) have shown that the rainfall-evaporation ratio ("moisture ratio") is dominant in determining the distribution of vegetation in the United States. The physiological problem as to why certain plants are enabled to withstand the severe conditions of drought, while others will survive only when the water supply is moderate, and still others only when it is abundant, has by no means been completely solved.

The general presumption has been that the ability of the plant to conserve water in the leaves through the internal conditions operative in those leaves is the important factor in drought resistance. Livingston (37) and Bakke (4) believed that the "foliar transpiring power" was a fairly accurate index of the ability of leaves to conserve water, and hence of the water relations of the plant. "Transpiring power" is a measure of the rate of water vapor loss from leaves as controlled by internal conditions. Bakke concluded from his experimental work that this method "———offers an apparently adequate and somewhat simple means for classifying plant forms in a scale of xerophytism or of mesophytism." Pool (47), however, in an extended investigation, found only indifferent correlation between habitat xerophytism and "transpiring power." The importance of foliar "transpiring power" as an index of relative xerophytism has apparently been overestimated by some of the pioneers in this field.

Variations in the resistance offered by leaves of different species to water vapor loss have been explained in the past principally on the basis of the anatomical features of the leaves, such as number, size, and distribution of stomata; epidermal hairs, cuticle, compactness of tissues, schlerenchyma, etc. Pool also attempted to correlate leaf structure with "transpiring power" but his work seems to show that leaf anatomy is an inadequate explanation of variations in the resistance of leaves of different species to transpirational water loss. Some structures commonly considered characteristic of xerophytic leaves may have a negligible effect upon transpiration. Sayre (49) has shown that the abundant epidermal hairs of the mullein (*Verbascum Thapsus*), for example, have no appreciable effect

in decreasing transpiration. Some plants with leaves apparently xeromorphic, such as *Ilex opaca* (holly), *Rhododendron maximum* (rhododendron), and *Cyrtomium falcatum* (holly fern), are in habitat mesophytic. On the other hand some plants will endure extreme drought whose leaves are lacking in any xeromorphic features. Such apparent anomalies may be largely cleared up by studies of the physical properties of leaves and leaf saps, and as microchemical and microphysical methods are developed, of the protoplasm.

Maximow (41) believed that the ability of the protoplasm of the leaf cells to endure the wilted condition is the essential factor in drought resistance. By the wilted condition is meant a semi-permanent diminution in the water content of the leaf cells. This is not necessarily a visible phenomenon, since in leaves with abundant ligneous tissue wilting may be obscured. According to this worker many xerophytes are known to have high transpiration rates when the water supply is favorable, while some mesophytes have low rates of transpiration. Transpiration is, therefore, not an adequate measure of relative xerophytism. The physico-chemical properties of the protoplasm and of the cell sap must be such that the protoplasm can endure or resist this deprivation of water sufficiently to prevent coagulation of the protoplasmic colloids. The problem of drought resistance, considered from this standpoint, resolves itself into a question of why the protoplasm in the leaf cells of certain species can endure the partial desiccation involved in wilting, while the protoplasm of other species cannot.

It is now generally agreed that during the formation of ice in plant tissues, water usually freezes first in the films on the outer surface of the cell walls, bounding the intercellular spaces. As the freezing process proceeds, water gradually moves from the interior of the cell into the cell walls, replacing that which was withdrawn from the walls by the process of crystallization. It is this loss of water from the cell which is the cause, directly or indirectly, of injuries accompanying the formation of ice crystals in the tissues of plants. Wiegand (54, 55) has given an excellent historical summary of the literature dealing with the formation of ice in plant tissues as a setting for his own investigations in this field. The similarity of the effects of drought and freezing on the cell are apparent; both are, from the standpoint of fundamental physiology, desiccation processes. It is probable, therefore, that some of the internal cell conditions important in drought resistance are also important in frost resistance.

The literature covering previous investigations of the causes of death during the freezing process, and of the bases of frost resistance in plants has been comprehensively reviewed by Abbe (1), Blackman (6), Chandler (7), Rosa (48), Harvey (32), and Newton (45). Only a condensed statement of the more pertinent contributions to our knowledge in these fields will be included here. Muller-Thurgau (43) and Molisch (42) believed that the injury involved in the freezing to death of a cell resulted from the withdrawal of water from the protoplasm, which resulted in its structural disorganization. Maximow (40) thought that death resulted only when the water was withdrawn from the plasma membrane. The withdrawal of water from the cell gradually concentrates the electrolytes in solution in the vacuole. Gorke (10) advanced the theory that this concentration of the electrolytes had a salting out effect on the proteins in the cell sap and protoplasm, and that this was the cause of death. Harvey (32) believed that this salting out effect is inadequate to explain the precipitation of the proteins upon the freezing of a plant juice unless the increase in the hydrogen ion concentration which accompanies a lowering of temperature is taken into consideration, as a contributing or modifying factor.

There are several conditions which may operate in the cell in such a way as to prevent injury in the freezing process. A high osmotic value of the cell sap, or a high force of imbibition in the protoplasmic colloids would tend to prevent movement of water from the cell when crystallization occurs in the intercellular spaces. Newton (45) found the latter condition to be an important factor in the frost resistance of winter hardy wheat. Disorganization of the protoplasmic complex due to the concentration of electrolytes accompanying the withdrawal of water from the cell may be prevented if the proteins have been converted to a less readily precipitated form during the hardening process (Schaffnit, 51; Harvey, 32). Accumulation of sugars in the cells exerts a protective action which checks or prevents the precipitation of proteins (Schaffnit, 51; Lidforss, 36). Maximow (40) has found that solutions of many non-toxic compounds, both organic and inorganic, which have a low eutectic point relative to the freezing point, exert a remarkable protective action on the freezing of plant tissues which are immersed in them.

This paper reports the results of an investigation upon the range of certain physical properties of leaves and leaf saps in fifty species of plants, representing many of the habitats common to central Ohio. Determinations were made of the water content of the leaves, amount of sap which could be expressed from the leaves under a standard treatment and pressure; and of the osmotic value, percent of solid matter, and colloidal content of the expressed leaf saps. One purpose of this general survey was to secure data upon the range of these physical properties in the leaves of native plants, and to discover the correlations, if any, between habitat or growth form and the physical properties of leaves. The native vegetation of Ohio presents many unsolved problems in the physiology of frost resistance, and in the physiology of relative drought resistance. It is hoped that this study may fulfill a second purpose by serving as a foundation for more detailed investigations upon some of these problems.

II.

REVIEW OF LITERATURE.

The only physical property dealt with in this paper which has been extensively studied by previous workers is the osmotic value* of the expressed leaf saps. The first critical work on the determination of the osmotic pressure of leaf saps by the cryoscopic method was that of Dixon and Atkins (8, 9) in 1913. They demonstrated the necessity of freezing or otherwise treating the leaf sample before the sap was expressed in order to obtain representative results. Their earlier work and the work of previous investigators had shown that samples of sap expressed from untreated tissues could not be regarded as typical because samples of sap successively extracted from the same tissue differed in osmotic pressure. This differential expression of the sap is almost completely eliminated if the leaf sample first be frozen.

Harris (16-30, inc.) and his collaborators have made a survey of the osmotic pressures of the expressed leaf tissue fluids of plants from a wide range of habitats. Osmotic pressures were

* A solution such as an expressed plant sap has only a potential osmotic pressure. In this paper this is termed the osmotic value. The term osmotic pressure is retained, however, when discussing the results of investigators who have used this term consistently in their writings.

calculated from the depression of the freezing point of saps expressed after freezing the tissue in an ice-salt mixture. These investigations have been important in determining the range of osmotic pressures in leaf tissue fluids. In general the expressed juices of leaves from plants growing in dry or saline habitats are higher in osmotic pressure than the leaf saps from plants growing where the moisture conditions of the soil are more favorable. Important exceptions to this generalization, the cacti for example, show the inadequacy of explanations of the water relations of plants on the basis of osmotic pressures alone. Ligneous plants, in general, have leaf saps with higher osmotic pressures than herbaceous plants. The osmotic pressure of the expressed sap of parasitic forms is generally, but not invariably, higher than that of the host plant. Epiphytes show lower osmotic pressures than terrestrial forms. Correlated studies upon the electrical conductivity of the expressed leaf saps have shown that this is higher in the tissue fluids of herbaceous species than in ligneous species.

Korstian's study (34) of the "sap densities" of plants in the Wasatch mountains of Utah and vicinity is a recent contribution in this field. The results of this investigation confirm in general those of Harris and his co-workers. Saps from herbaceous plants were found to have lower osmotic values than saps from ligneous species. The sap of shade plants showed lower values than that of sun plants of the same species. Young leaves and shoots showed lower sap concentrations than older tissues. Plants from dry or saline habitats showed a higher concentration of solutes in the expressed leaf saps than plants from habitats where the moisture conditions were more favorable. Correlated studies were made of temperature, moisture content of the soil, and evaporation. The opinion of this investigator is that "—— the sap density may be used as an index of site in correlating the great complex of environmental factors with the physiological responses of the plant." The osmotic value of the sap of a species is not constant. It may be influenced by any of the environmental conditions affecting transpiration, absorption, and food manufacture. The osmotic value of the sap of plants was found to be affected more by fluctuations in the moisture conditions of the habitat than by fluctuations in temperature or light.

III.

METHODS.

1. *Collection and Preparation of Leaf Samples.*

The leaf samples were all collected between the hours of one and three in the afternoon, with the exception of those in Series III, which were collected between eleven and twelve in the morning. The leaf samples were placed, on collection, in thick walled test tubes, one and one-quarter inches in diameter, and eight inches long. The capacity of such a tube is about fifty grams of leaves. Two tubes, or approximately one hundred grams of leaves, were collected for each species. Except when composed of very large leaves, as, for example those of the water lily, such a sample consists of several hundred leaves and this number should be sufficient to insure representative results. Most samples of tree leaves were collected from one individual. From herbs, shrubs, and a few of the smaller species of trees it was impossible to secure an adequate sample from one plant. Whenever this was the case, care was taken to collect the leaf material from individuals growing in the same habitat, and, whenever possible, from closely adjacent plants.

Certain almost obvious precautions were taken in the selection of the leaf samples. Only the blade of the leaf, or if the leaf were compound, only the leaflet blades were collected. When the mid-vein was extremely prominent, as in the leaves of burdock (*Arctium minus*), dock (*Rumex patientia*), and water lily (*Castalia odorata*), it was removed; otherwise the entire leaf blade was used. Only apparently sound and healthy leaves, with a uniform green color, were chosen. Collections were made only when the external leaf surfaces were free from moisture. Samples of evergreen leaves were selected only from leaves produced during the current season's growth.

The leaf samples, collected as described, were used as material for all the determinations except that of the total moisture content of the leaves. The filled test tubes were stoppered tightly with rubber stoppers immediately after the collection of the sample and plunged at once into a freezing mixture of ice and salt at a temperature of about -20°C . The tubes were arranged in the freezing bath so that the stoppers were emerged, thus avoiding possible contamination by the ice-salt mixture. Double-walled, galvanized iron buckets, in-

sulated with cotton batting, were found serviceable as containers for the freezing mixture. The tubes were left in the ice-salt mixture from fifteen to twenty hours, in practice usually overnight. If necessary the freezing mixture was renewed.

The effect of freezing the leaf tissue is to increase the yield of sap when expressed under pressure. The sap sample is also more representative when this method is used. Dixon and Atkins (8), while working on means of extracting sap for osmotic pressure measurements, developed this method. These investigators froze the samples in liquid air. Gortner and Harris (11), also primarily concerned with osmotic pressure measurements, substituted the ice-salt bath as a more universally available mode of freezing. The method used in this investigation is essentially that developed by them. Previous workers (André, 2; Marie and Gatin, 39) had shown that successive portions of saps expressed under pressure from unfrozen tissues showed progressively increasing concentrations of solutes as measured by the depression of the freezing point. The following explanation of such results was given by Dixon and Atkins. The initial pressure forces out practically pure water through the differentially permeable cell membranes, which are freely permeable to water, but difficultly permeable to most of the solutes present in the cell sap. With increasing pressure some of the cells burst, contributing their entire complement of sap, as well as their protoplasmic contents to the portion of the sap which has already been expressed. The sap released by the bursting of the cells will contain a higher proportion of solutes than that which is forced out through the protoplasmic membranes. Increase in the pressure will cause the bursting of additional cells, therefore each successive sample will contain more solutes, and will consequently have a higher osmotic pressure. If, however, according to these workers, the tissue first be frozen, the membranes become much more freely permeable; solutes as well as water are expressed, and a more representative, as well as a larger sample is obtained. Furthermore, successive portions of sap expressed from the frozen tissues used by these investigators did not show the increasing concentrations of solutes which occurs when unfrozen tissues are used. Gortner, Lawrence and Harris (12) have shown that successive portions of sap expressed from unfrozen tissues may show decreasing concentrations of solutes, or all of the fractions may show approximately the same concentration of solutes. Newton,

Brown and Martin (46) have shown that there are slight variations in the freezing point depression (hence in the osmotic value) of successive portions of sap expressed from frozen wheat leaves. This variation is much less when the sap is expressed from frozen samples of the leaves than when it is expressed from unfrozen samples, and these investigators point out the fact that the difference in the behavior of these two samples is merely one of degree. These extensions of the results of Dixon and Atkins do not invalidate the freezing method as yielding the most representative results when the osmotic value of the expressed sap is to be measured.

There can be little doubt that the increase in permeability of the cells after the freezing treatment is due to the actual destruction of the cytoplasmic membranes and consequent death of the cells. Deciduous leaves and the leaves of evergreens in the summer condition uniformly show a greater yield of sap from frozen than from unfrozen tissues, showing that some or all of the cells in the leaves have been affected by the freezing treatment.

Knudson and Ginsberg (33) reported that the expressed tissue fluids from the leaves of *Iresine Herbstii* Hook. showed about the same value for osmotic pressure when they were prefrozen in the ice-salt mixture as when the preliminary treatment consisted in freezing them in liquid air. It would be dangerous to attempt to generalize this result to other species, however, until more data are available.

Another advantage of the prefreezing treatment is that there is little likelihood of any change taking place in the sample after it is once frozen. When samples must be collected at considerable distances from the laboratory, as was the case in this investigation, it is the only method which may be used with any assurance that considerable physical and chemical changes have not taken place in them before they can be used in experimental work.

Tubes containing frozen leaf samples were thawed by exposing to room temperature for about fifteen minutes. All samples were weighed to determine the exact amount of leaf material used in each determination. The weighing was carried out in the tubes in which the sample was collected. After weighing the tubes were kept in ice water for the short interval of time which elapsed before the sap was expressed.

2. *Expression of the Leaf Tissue Fluids.*

Knudson and Ginsberg (33) have demonstrated experimentally that the osmotic value of plant juices expressed from frozen leaf tissues varies with the pressure used in extraction. The concentration of solutes in a plant sap is dependent upon the pressure used in extracting it, and the physical and chemical properties of that sap will vary accordingly. It is important that the extractions of plant saps be made under known pressures. This standardization will make possible a duplication of a determination at any time or place that measured pressures are available. Newton, Brown, and Martin (46) have also emphasized the importance of standardizing the extraction procedure, particularly with respect to the pressure used. A press similar in design to that originated by Knudson and Ginsberg was first used. This consists of a cylinder and tightly fitting piston with a delivery pipe at the base. For the dimensions and details of the construction of this piece of apparatus, the original paper should be consulted.

The method of using this press will be briefly described. A fifteen inch square of linen cloth was folded twice and placed in the bottom of the cylinder. The sample of leaves was transferred from the tubes in which it was collected and frozen, and placed on the cloth in the bottom of the press cylinder. The cloth was drawn up around the sample and the piston inserted. Pressure was applied to the top of the piston by means of a materials testing machine* and gradually brought up to five thousand pounds to the square inch. It should be noted that the pressure was merely brought up to five thousand pounds and not maintained at that figure after it once had been attained. After the maximum has been attained there is a slow diminution in the pressure to which the leaf sample is subjected, due to the gradual exudation of sap through the delivery pipe. A uniform practice was followed of allowing the press to drain for five minutes after the maximum pressure had been attained. In some of the earlier determinations muslin cloth was used instead of linen, but the tensile strength of the muslin was not always sufficient to withstand the pressures used, consequently it frequently ruptured with an accompany-

* The writer is indebted to Professor Horace Judd, of the Department of Mechanical Engineering of the Ohio State University, for permission to use this machine.

ing extrusion of leaf material through the delivery pipe. This style of press proved heavy and cumbersome for general work. Furthermore, the piston frequently became stuck in the cylinder, and could only be removed by mechanical means, an awkward and tedious procedure. The atmospheric pressure on a piston with this top area (12.56 square inches) is about one hundred and ninety pounds and this is sufficient to prevent its removal by hand whenever the leaf sample becomes tightly enough wadded into the bottom of the press cylinder to prevent the ingress of air through the delivery pipe. For these reasons a smaller and improved press was designed for these investigations. The essential design is similar to that of the press previously employed. The piston and cylinder were turned from steel and ground to fit within .0015 inch. The piston is five and one-half inches high and three inches in diameter. The cylinder is six inches high and has an outside diameter of four inches. Its inside diameter is three inches, and it is bored out to a depth of four and one-half inches.

A groove cut around the inside wall of the cylinder one inch from the top was designed to act as a liquid seal and prevent the upward movement of sap past that point. A groove was also cut around the base of the cylinder, adjacent to the vertical wall. This was designed to collect the sap and feed it to the two delivery pipes on opposite sides of the cylinder. These tubes were made of brass and threaded into place so that they might easily be removed for cleaning. An important addition to the press is a metal disk one-quarter inch thick, of a diameter slightly smaller than the inside diameter of the cylinder. On the lower side of this disk are eight equally spaced radiating grooves, one-eighth inch wide and one-eighth inch deep. Six one-sixteenth inch holes, spaced at equal intervals, are drilled through the metal disk into each groove. The removal of both the piston and the metal disk from the cylinder is facilitated by means of handles which screw into place. A press of these dimensions will readily accommodate a hundred gram leaf sample.

The employment of the metal disk described above makes it possible to practically dispense with the use of cloth for wrapping the samples. Two or three disks of linen cloth, cut to a slightly larger diameter than the cylinder, and placed on top of the metal disk are all that is required. In fact, when leaves containing considerable amounts of ligneous tissue are used, the linen disks can be dispensed with. The leaf sample is placed

directly on top of the cloth disks. The elimination of cloth for wrapping the sample is one of the advantages of the re-designed press. It is also convenient in that it is small, light and easily handled, and that all parts may be readily cleaned. If used with reasonable care, the piston never sticks in the cylinder. This type of press is well adapted for the collection of samples of expressed sap at successively increasing pressures and for determining pressure dehydration curves for plant tissues. It may also be used to determine the pressure necessary to express a given quantity of sap. Presses of this design may be built of any convenient size. They are superior to any type within the writer's experience for general work in the expression of plant tissue fluids.

The expressed plant juices were still cool as they came from the press. They were collected in porcelain evaporating dishes, poured immediately into graduated test tubes, and kept in ice water until used for further determinations. Of course it is impossible to maintain the integrity of an expressed leaf fluid for even a second after it is forced from the tissues. Oxidations occur as soon as the fluid comes in contact with the air, sometimes accompanied by a conspicuous darkening of the sap. Internal changes in such a complex system of physical and chemical equilibria as a plant sap are bound to occur. This is particularly true since many of the compounds brought together in the sap were separate in the leaf tissues. Chemical and physical changes in the saps were minimized as far as possible by keeping them in stoppered tubes in ice water. Furthermore, determinations of the properties of the saps were made as soon as practicable after their extraction. The time intervening between the expression of the sap and the completion of determinations upon that sap was never more than four hours, and was usually much less. Tests made of the osmotic value of several saps kept under these conditions showed that no appreciable change in this property, at least, occurred within this period of time.

Equal weights of leaves from different species of plants showed marked differences in the yield of sap. The range, for a hundred gram sample was from two cubic centimeters in *Tsuga canadensis* (hemlock) to sixty-seven cubic centimeters in *Bryophyllum calycinum*. The exact amount of juice expressed was measured as accurately as feasible. The delivery pipes were unscrewed and drained. When linen cloth was used for

wrapping the samples before placing them in the press, the amount of water absorbed by the cloth was determined by drying in an oven at 103°C. No appreciable error is incurred by considering the weight of the water evaporated as equal to the amount of sap which had been absorbed by the cloth. Even when the redesigned press was employed and the only cloth used consisted of several linen disks, this correction was made. The amount of sap absorbed by three of these disks, the number usually employed, seldom exceeds one cubic centimeter, however, and for many practical purposes can be disregarded.

The fact that equal sized samples of different species of leaves yielded varying amounts of sap when subjected to the same treatment and pressure made it important to carry out determinations of the total water content of each leaf tissue. With these data available it is possible to determine how much of this variation in the amount of water which can be expressed is due to variations in the water content of the leaf tissues, and how much of it is due to the resistance of those tissues to dehydration under pressure. The total water content of the leaves was determined by drying approximately twenty-five gram samples of leaf tissue to constant weight in an oven at 103°C.

3. Determination of the Osmotic Value of the Expressed Leaf Saps.

The determination of the depression of the freezing point of the expressed leaf juices was made by the classical method originated by Beckmann (5). Gortner and Harris (11) have discussed the application of this method to plant saps and have suggested several modifications in apparatus and technique which greatly facilitate the determination. Several of these suggestions were followed in the present work; insulation of the freezing bath, use of a metal air jacket, and employment of auxiliary freezing baths.

A double-walled, galvanized iron can, insulated with cotton batting, makes a convenient freezing bath. This bucket is seven inches high, three inches in internal diameter, and has a space between the walls of one and one-half inches. A metal cover is also provided through which is inserted a stopper holding a metal air jacket. A brass tube, one and one-eighth inches in internal diameter, and about six inches long, stoppered

at the bottom, makes a satisfactory air jacket. This air jacket is plugged with a rubber stopper through which is inserted a hard glass freezing tube. Culture tubes, 18 x 150 mm. are well adapted for this purpose. A Haidenhain thermometer was used with this freezing point apparatus. It is less cumbersome than the more familiar Beckmann type and the smaller size of the bulb permits the use of smaller freezing tubes and smaller samples of sap. As little as four cubic centimeters of sap will suffice with this arrangement. Moreover, the Haidenhain thermometer is less susceptible to change in the zero point than the standard Beckmann type*. This slow change has probably been correctly explained by Atkins (3) as due to the distillation of mercury from the curved surface of the hanging drop at the top of the reservoir of the Beckmann thermometer to the flat surface at the lower end. It is well known that the vapor pressure of a convex surface is greater than that of a flat surface. In the Haidenhain model there is only one mercury surface so that such a distillation with a consequent change in the zero point of the thermometer is impossible. There are slight changes in the zero point of this type, however, due probably to minute fluctuations in the volume of the thermometer bulb, probably due, in part, at least, to aging of the glass. For this reason the freezing point of distilled water was checked every few days throughout the season's determinations. The variation in three months was found to be less than .01 degree. The freezing tube is also provided with a rubber stopper and stirring rod. A stirring rod may also be provided for the freezing bath if desired, but the insulation makes this unnecessary.

In practice, several auxiliary baths were used, depending upon the number of determinations to be made. These baths were water-ice-salt mixtures, cooled to -1° or -2°C . Wide-mouthed thermos bottles (internal diameter two inches) make very satisfactory containers for these auxiliary baths. About five cubic centimeters of sap, centrifuged to remove suspended debris, were poured into a freezing tube, a thermometer and a stirring rod inserted through the stopper, and the tube placed in one of the pre-cooling baths. Several samples may be cooling at one time. After the mercury had fallen into the capillary, the freezing tube and attached thermometer were transferred to the freezing bath. This may be at a temperature of -10 to -15

* Improved models of the Beckmann thermometer are now on the market of such construction that change in the zero point is at a minimum.

degrees. The fact that the mercury is already low in the capillary and that the freezing tube is surrounded by a jacket of air permits the use of such a low temperature. The sample was slowly stirred until undercooling and subsequent freezing had taken place. A thermometer reading must be taken both for the point of maximum undercooling (convergence temperature) and for the freezing point. Rapid agitation of the sap sample with the stirring rod was sometimes resorted to to induce crystallization. Usually, however, this was unnecessary. With this technique, after a little practice, from five to six determinations may be made in one hour. Between determinations the metal air jacket was kept plugged with a rubber stopper in order to keep the air at a low temperature. It is also advisable to provide the air jacket with a thermometer so that a check may be kept on its exact temperature.

Observed freezing point depressions were corrected for undercooling by the formula of Harris and Gortner $D = D' - .0125uD'$ (14). Harris (31) has recently expanded it into a table which greatly facilitates corrections for undercooling. The corrected freezing point depression readings were converted into equivalent osmotic values from Harris and Gortner's tables (14, 15). The direct relation between the freezing point and the osmotic pressure is expressed by the equation $O.P = 12.06D - 0.021D^2$, (Lewis 35). The table cited is based on this formula.

4. *Determination of the Percent of Solid Matter and the Colloidal Content of the Leaf Saps.*

Gortner and Hoffman (13) were the first to point out that the colloidal content of a plant sap may be an important physical property. A method for determining the relative amount of hydrophilic colloids present in plant saps has been introduced by Newton and Gortner (44). This method expresses the colloidal content in terms of "bound water". The method will be briefly reviewed, but for details the original paper should be consulted.

The solid matter content of the sap is first obtained by the refractometer method of Gortner and Hoffman (13). An Abbe-Hilgard refractometer was used*. The percentage of water in the sap was obtained from published tables of the United States Bureau of Standards (56), and the total solids calculated

* Lent to the writer by courtesy of Dr. Alpheus W. Smith, of the Department of Physics of the Ohio State University.

from these data. This method seems to be at least as accurate as the alternative method of desiccation *in vacuo*, and is more economical of time and apparatus.

The freezing point depression of the sap is determined. A fresh sample of the sap containing exactly ten grams of water is then weighed out. To this is added 3.422 grams of sucrose, just sufficient to make a weight molecular solution. The sucrose crystals are first ground fine in a mortar to insure rapid solution. The freezing point depression of a weight molecular solution of sucrose is 2.085 degrees. This value is in excess of the usual molecular freezing point depression (1.86) because of the hydration of the sucrose molecules and consequent reduction in the amount of available solvent. Six molecules of water are bound to each molecule of sucrose (Scatchard, 50). Hence one mol of sucrose binds six mols or 108 grams of water. The net result is one mol of hydrated sucrose dissolved in 892 grams of water. Since this is more concentrated than a molecular solution the freezing point depression is greater than the molecular lowering. After the addition and solution of the the sucrose, the freezing point depression of the sap sample is redetermined. This value is usually found to be greater than the original freezing point depression plus 2.085. This excess depression is considered to be due to the fact that part of the water is "bound" by hydrophilic colloids present in the sap and is thus unavailable as a solvent. That is, the sucrose solution is more concentrated than a weight molecular solution because the 8.92 grams of water not bound by the sucrose are not all free to act as a solvent, and consequently the freezing point depression of the solution is greater.

The percent of bound water is calculated as follows:

$$\text{Bound water} = \frac{D' - (D + 2.085)}{D' - D} \times 89.2.$$

D is the freezing point depression of the expressed juice, D' is the freezing point depression after the addition of the sucrose, 2.085 is the molecular freezing point depression for sucrose, and 89.2 is the percentage of free water in a weight molecular solution of sucrose.

This method is adapted to numerous modifications in technique. In general, it consists in producing a known change in the concentration of a solution for which the depression of the freezing point is known. The resulting change in the depression

of the freezing point is measured and compared with the change calculated for the known change in concentration. If the depression of the freezing point is greater than the theoretical expectation, it measures the amount of water unavailable for solution, i. e. the bound water.

If the bound water be absorbed, as Newton and Gortner's work seems to indicate, this method should give an approximate measure of the surface area of the colloids available for absorption. It is not a measure of the mass of material present in the sap in the hydrophilic colloid state, as the same mass may have different surface areas depending upon the degree of dispersion and shape of the particles.

The possible sources of error in this method have been discussed by its originators. The validity of the method as a measure of the hydrophilic colloid content of a sap rests upon the assumption that there are no other substances present in the sap which bind appreciable amounts of water. The only non-colloidal substance which is likely to be present in plant saps in such amounts as to bind appreciable amounts of water is sucrose. An initial concentration of sucrose in the sap equal to a five percent solution, for example, would bind about 1.6% of the water present. This possible source of error is not emphasized by the originators of this method, but in the interpretation of the results of an investigation covering a variety of plant saps it should be borne in mind.

IV.

THE RANGE OF PHYSICAL PROPERTIES IN THE LEAVES OF NATIVE OHIO PLANTS.

This investigation was conducted during the summer of 1925. Leaf samples were taken from about fifty species of plants, practically all of which are native to central Ohio. Determinations were made of the total water content of the leaves, the amount of sap expressed from the leaves under a standard treatment and pressure, and of the osmotic value, percent of solid matter, and colloidal content of the expressed leaf juices. A standard method of freezing all samples in an ice-salt mixture overnight, and expressing the sap under 5000 pounds of pressure was followed, as described in the preceding section. Unless this pre-freezing and high-pressure extraction method were used, the leaves of many of the ligneous species

investigated failed to yield sufficient volumes of sap to permit these determinations. This method unquestionably yields representative results for measurements of the osmotic value and solid matter content of the expressed tissue fluids. The pre-freezing treatment presumably has a precipitating effect on the cell proteins, and this source of error may affect the determination of the colloidal content of the sap.

A summary of the data resulting from these determinations is presented in Table 1. Series I represents plants from a mixed mesophytic ravine forest and an adjacent oak hickory forest near Columbus. Series II consists of plants from near Sugar Grove, Ohio. Leaves of *Rhododendron maximum* (rhododendron) and *Tsuga canadensis* (hemlock) were collected from the north facing slope of a deep, moist ravine. This habitat is abundantly supplied with soil water. All the other plants in this series grew on the top of a high, well-drained ridge. This was the driest habitat studied. This ridge is forested with an open forest of mixed *Pinus rigida* (pitch pine) and *Pinus virginiana* (scrub pine), which is evidently the pioneer tree association in a secondary succession. The shrubs and younger trees are all deciduous with the exception of the broad leafed evergreen, *Kalmia latifolia* (mountain laurel). Series III was taken from the banks of the Big Walnut Creek near Columbus. Series IV represents bog plants and hydrophytes from Buckeye Lake and the associated bog. *Alnus rugosa* (smooth alder), *Pyrus arbutifolia* (chokeberry), *Vaccinium macrocarpon* (cranberry), *Gaylussacia baccata* (black huckleberry), and *Osmunda cinnamomea* (cinnamon fern) were taken from the bog proper. This bog is surrounded by the artificially formed Buckeye Lake and the remaining plants in the series, with the exception of *Castalia odorata* (water lily), grow on the margin between the bog and lake. They are typically pond or swamp margin plants. *Castalia* was taken as an example of a submerged hydrophyte with floating leaves. Series V consists of some common herbaceous weeds. Series VI was taken from the garden and Series VII from the greenhouse of the Department of Botany.

The date of collection is given for each sample. The columns of data, in order, represent the percentage of water in the leaves, the number of cubic centimeters of sap expressed per hundred grams of sample under a pressure of 5000 pounds per square inch, the percent of the total water present which is expressed

as sap under this pressure, the osmotic value of the expressed sap in atmospheres, the percentage of total solids in the expressed sap, and the percentage of bound water in the expressed sap. Blanks appear in the table only where the volume of sap obtained proved inadequate for the missing determinations, or where accidents occurred to the samples.

Discussion will be restricted largely to the range of these physical properties in the species, and over the variety of habitats studied. The water content of the leaves ranges from 51.9% in *Fagus grandifolia* (beech) to 92.7% in *Bryophyllum calycinum*. The averages for the plants studied are trees 60.1%, shrubs 63.7%, and herbs (exclusive of the two species taken from the greenhouse) 76.4%.

The number of cubic centimeters of sap which could be expressed from one hundred grams of leaf tissue under a pressure of 5000 pounds to the square inch varied from two in *Tsuga canadensis* (hemlock) to sixty-eight in *Bryophyllum calycinum*.

That the volume of water expressed is based, in part, at least, on other factors than the water content of the leaves is shown by the fact that the percent of the total water which can be expressed as sap shows a remarkable variation among the different species studied. The range is from 3.4% in *Tsuga* to 88.0% in *Helianthus*. The volume of water which can be expressed from a sample of leaves by pressure may have an important physiological significance. This volume will depend upon the water content of the leaf tissues, the mechanical resistance offered by the tissues to pressure, and the affinity of the tissues for water. In studies of seasonal variations in the amounts of sap which can be expressed from mature leaves, variations in the first two of these factors are frequently so small as to be negligible. Newton (45) found that it was impossible to express appreciable amounts of sap from pre-frozen samples of winter hardy wheat during the winter months. During the summer, however, relatively large amounts could be expressed. The writer has found a similar seasonal variation in the amounts of sap which can be expressed from the leaves of native Ohio evergreens. The data on which this statement is based will be published in a subsequent paper. It seems clear, as Newton has pointed out, that this phenomenon must be due to seasonal shifts in the amount and physical condition of the intracellular colloids. It is his belief that larger amounts of water are bound by these colloids in the winter than in the

summer, due probably to the fact that most of the cell colloids are in the gel condition during the winter months. This is probably an important factor in the frost resistance of species with evergreen leaves. Young leaves, in which vacuolation of the cells is not yet complete, should offer a greater resistance to the expression of sap under pressure than mature leaves, since larger proportions of the water in the cells of young leaves are bound by molecular forces. This may account for the fact that the young leaves of some species are more resistant to freezing than mature leaves. No critical experimentation has yet been carried out upon the relative resistance offered by young and mature leaves to the expression of sap under pressure.

The data on osmotic value corroborate the results of earlier investigators in regard to the relation between this property and growth form. Trees in general have higher osmotic values for the expressed leaf sap than herbs, and shrubs occupy an intermediate position. The three groups are by no means mutually exclusive, and for some herbs the osmotic value of the expressed sap was found to be almost as high as the most extreme found in trees. The average for herbs, exclusive of those taken from the greenhouse, is 13.6 atmospheres, for shrubs 16.0 atmospheres, and for trees 17.2 atmospheres. The range for ligneous species is from 11.79 atmospheres in *Rhododendron maximum* (rhododendron) to 21.30 atmospheres in *Populus deltoides* (cottonwood). There is no marked correlation of the osmotic value of the expressed leaf sap with habitat, and Korstian's conception of such a relation is not substantiated. Of course it should be recognized that the extremes of habitat represented in the Ohio flora do not approach the extremes found in Utah, where Korstian's work was done. On the other hand there is clearly a marked difference in terms of environmental factors of a habitat on the margin of a stream, represented by Series III, and one at the summit of a well drained and usually dry ridge, represented by most of the plants in Series II. Yet the two habitats are scarcely distinguishable on the basis of the osmotic values of the expressed leaf saps. The determinations for the trees of the dry ridge average slightly more than one atmosphere higher than the determinations for the trees along the stream margin. Unless differences in the habitat factors are more extreme than the differences between the habitats in central Ohio the osmotic value of the expressed leaf sap is apparently not a safe criterion of site.

The percent of total solids in the expressed saps shows a range of from 1.3% in *Zebrina pendula* (wandering jew) to 20.15% in *Rhus copallina* (dwarf sumac). The average for the plants studied in this investigation are 8.0% for herbs, 12.8% for shrubs, and 13.0% for trees. It appears that trees and shrubs, in general have a higher percent of solid matter in the expressed leaf sap than herbaceous plants.

The percentage of bound water in the expressed leaf tissue fluids ranges from none in *Castalia odorata* (water lily), *Osmunda cinnamomea* (cinnamon fern), and *Zea mais* (corn) to 23.4% in *Pinus rigida* (pitch pine). The average for the plants studied in this investigation are 12.6% for trees, 8.7% for shrubs, and 5.7% for herbs. The average for trees is distinctly higher than that for shrubs, and the average for shrubs is, in turn, markedly higher than that for herbs. The range of variability of this physical property of the expressed saps within each of these three groups is greater than for the other two physical properties of the sap studied. Apparently there is no correlation between the percent of bound water in the sap and the habitat.

It is clear, that over the range of habitats studied, the physical properties of the leaves are more closely correlated with growth form than with the factors of the environment. Herbaceous plants, in general, have a higher leaf water content than ligneous plants. The sap expressed from the leaves of herbaceous plants usually shows a lower osmotic value, a lower percentage of solid matter, and a smaller percent of bound water than the sap expressed from the leaves of ligneous plants. Shrubs usually occupy a position intermediate between trees and shrubs in regard to these measurements.

TABLE I.
SUMMARY OF THE PHYSICAL PROPERTIES OF LEAVES AND LEAF SAPS.

	Date	Plant	Water Content of Leaves, Percent	Sap Yield cc. per 100 Grams	Percent of Total Water Expressed	Osmotic Value of Sap in Atmos- pheres	Total Solids Percent	"Bound Water" Percent
		SERIES I.						
1	Aug. 3	Quercus alba.....	56.1	24	42.7	20.39	14.3	0.9
2	Aug. 6	Quercus imbricaria.....	55.7	6	10.7			
3	Aug. 12	Celastrus scandens.....	76.4	59	77.2	16.26	10.7	11.4
4	Aug. 12	Cercis canadensis.....	71.1	40	56.2	12.52	7.5	11.6
5	Aug. 12	Benzoin aestivale.....	77.7	54	69.4	13.06	5.7	6.3
6	Aug. 12	Quercus velutina.....	56.9	7	12.3			
7	Aug. 30	Fagus grandifolia.....	51.9	18	34.6	18.17	11.2	
8	Sept. 18	Acer rubrum.....	55.8	27	48.3	17.27	15.5	7.4
9	Sept. 14	Acer saccharum var. nigrum.....	58.2	28	48.1			
10	Sept. 14	Sassafras variifolium.....	61.4	23	37.4	20.42	16.8	10.8
11	Sept. 14	Carya glabra.....	57.3	18	31.4	19.74		
		SERIES II.						
12	Aug. 5	Rhododendron maximum.....	62.0	44	71.0	11.79	8.8	5.1
13	Sept. 15	Tsuga canadensis.....	57.9	2	3.4			
14	Aug. 8	Kalmia latifolia.....	55.7	21	37.7	18.14	13.1	8.6
15	Aug. 8	Acer rubrum.....	58.4	25	42.8	15.72	12.9	5.8
16	Aug. 8	Oxydendrum arboreum.....	75.2	53	70.4	18.69	15.0	18.4
17	Aug. 23	Gaultheria procumbens.....	62.0	32	61.5	15.06	11.5	7.5
18	Aug. 23	Smilax glauca.....	82.7	44	53.2	18.40	12.0	6.4
19	Aug. 23	Rhus copallina.....	63.0	45	71.4	19.02	20.1	4.3
20	Aug. 23	Cornus florida.....	64.8	45	69.4	17.34	11.9	8.7
21	Sept. 15	Pinus rigida.....	66.2	45	67.9	18.21	14.8	23.4
22	Sept. 18	Acer rubrum.....	56.5	30	53.0	15.04	14.9	6.1
23	Sept. 24	Sassafras variifolium.....	70.9	25	31.7	15.22		
24	Sept. 24	Castanea dentata.....	59.9	16	26.7		11.1	

SERIES III.									
25	Aug. 20	Acer saccharinum.....	58.3	27	46.3	14.54	11.9	5.0	
26	Aug. 20	Salix alba.....	63.3	36	56.8	14.22	11.4	10.2	
27	Aug. 20	Platanus occidentalis.....	65.0	45		12.08	8.2	5.2	
28	Sept. 14	Populus deltoides.....		43	66.1	21.30	16.6	10.3	
SERIES IV.									
29	Aug. 17	Alnus rugosa.....	55.1	29	52.6	17.51	13.6	6.9	
30	Aug. 17	Pyrus arbutifolia.....	59.2	6	10.1				
31	Aug. 26	Vaccinium macrocarpon.....	62.4	40	76.3	19.22	17.5	12.8	
32	Aug. 26	Gaylussacia baccata.....	55.7	33	59.2	19.08	17.4	9.8	
33	Aug. 26	Osmunda cinnamomea.....	74.9	55	73.4	14.88	8.4	0.0	
34	Aug. 26	Hibiscus Moscheutos.....	68.7	48	69.8	12.98	9.8	10.6	
35	Sept. 20	Decodon verticillatus.....	78.1	40	51.2	14.18	7.8	9.8	
36	Sept. 20	Sagittaria latifolia.....	81.5	30	36.8	13.24	7.7	7.3	
37	Sept. 20	Typha latifolia.....	77.9	24	30.8				
38	Sept. 20	Polygonum amphibian.....	71.9	54	75.1	11.80	7.1	5.3	
39	Aug. 26	Castalia odorata.....	75.9	64	74.5	14.74	8.4	0.0	
SERIES V.									
40	July 16	Taraxacum officinale.....	87.8			13.58	5.2	5.1	
41	July 19	Abutilon Theophrasti.....	68.2			13.04	9.4	3.6	
42	July 23	Saponaria offinalis.....	78.8			13.35	7.6	11.8	
43	July 23	Arcinum minus.....	87.0			10.84		11.2	
44	July 24	Rumex Patientia.....	91.6			6.68	3.4	6.9	
45	Sept. 14	Verbascum Thapsus.....	71.3	51	71.0	13.42	12.5	1.8	
46	Sept. 18	Poa pratensis.....	77.6	64	82.4	13.60	5.1	3.8	
SERIES VI.									
47	Aug. 30	Helianthus annuus.....	62.5	55	88.0	18.82	8.1	7.8	
48	Aug. 30	Zea mais.....	68.2	48	70.3	19.31	11.1	0.0	
SERIES VII.									
49	July 20	Zebrina pendula.....	89.2			4.81	1.3	5.0	
50	Nov. 9	Bryophyllum calycinum.....	92.7	68	72.8	5.79	4.5	2.3	

V.

SUMMARY.

1. A study has been made of the range of certain physical properties of the leaves and of the expressed leaf saps from plants native to central Ohio. Determinations have been made of the water content of the leaves, the yield of sap from the leaves under standard treatment and pressure; and of the osmotic value, solid matter content, and colloidal content of the expressed leaf saps.

2. A specially constructed press of the cylinder and piston type is described which involves certain new principals in design. This press has given satisfactory results for the expression of leaf tissue fluids from a variety of plants.

3. The numerical range of the physical properties of leaves and expressed leaf saps has been adequately summarized in Table I. The physical properties of the leaves of any species may be regarded as taxonomic characters, variable, of course, within certain limits depending upon the environment of the plants, and the maturity of the leaves.

4. No general correlation between the physical properties of leaves and habitat could be discovered. The osmotic value of the expressed leaf sap cannot be taken as a safe criterion of site over the range of habitats found in central Ohio.

5. The physical properties of the leaves of herbaceous plants as a class are quite different from those of ligneous plants as a class. The leaves of herbs usually have a higher water content than the leaves of ligneous plants. Sap expressed from the leaves of herbs usually has a lower osmotic value, lower solid matter content, and a lower colloid content (measured as bound water) than sap expressed from the leaves of trees and shrubs. Shrubs, in general, show values for these physical properties intermediate between those for trees and herbs.

The writer wishes to acknowledge his indebtedness to Dr. E. N. Transeau, Dr. H. C. Sampson, and Dr. J. D. Sayre, all of the Ohio State University, for helpful suggestions during the progress of this investigation, and for valuable criticisms of the completed manuscript.

VI.

LITERATURE CITED.

1. ABBE, C. Influence of cold on plants, a résumé. *Exp. Sta. Record* Vol. 6. p. 777. 1895.
2. ANDRE, G. Sur la composition des liquides qui circulent dans la végétale. *Compt. Rend. Acad. Sci. (Paris)* 142: 106-108. 1906.
3. ATKINS, W. G. R. Osmotic pressures of the blood and eggs of birds. *Sci. Pro. Roy. Dub. Soc. n. s. 12*: 123-30. 1910.
4. BAAKE, A. L. Studies on the transpiring power of plants as indicated by the method of standardized hygrometric paper. *Jour. Ecol.* 2: 145-173. 1914.
5. BECKMANN, E. Ueber die Methode der Molecularwichtsbestimmung durch Gefrierpunktniedrigung. *Ztschr. Phys. Chem.* 2: 638-45. 1888.
6. BLACKMAN, F. F. Vegetation and frost. *New Phytol.* 8: 354-62. 1909.
7. CHANDLER, W. H. The killing of plant tissue by low temperature. *Mo. Agr. Exp. Sta. Res. Bul.* 8: 143-309. 1913.
8. DIXON, H. H., and ATKINS, W. G. R. Osmotic pressures in plants. I. Methods of extracting sap from plant organs. *Sci. Pro. Roy. Dub. Soc. n. s. 13*: 422-33. 1913.
9. ———, and ATKINS, W. G. R. Osmotic pressures in plants. II. Cryoscopic and conductivity measurements on some vegetable saps. *Sci. Pro. Roy. Dub. Soc. n. s. 13*: 436-40. 1913.
10. GORKE, H. Ueber Chemische Vorgaenge beim Erfrieren der Pflanzen. *Landw. Vers. Stat.* 65: 149-60. 1906.
11. GORTNER, R. A., and HARRIS, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. *Plant World* 17: 49-53. 1914.
12. ———, LAWRENCE, J. V., and HARRIS, J. A. The extraction of sap from plant tissues by pressure. *Biochem. Bul.* 5: 139-42. 1916.
13. ———, and HOFFMAN, W. Determination of the moisture content of expressed plant tissue fluids. *Bot. Gaz.* 74: 442-46. 1922.
14. HARRIS, J. A., and GORTNER, R. A. Notes on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point. *Amer. Jour. Bot.* 1: 75-78. 1914.
15. ————An extension to 5.99° of tables to determine the osmotic pressure of expressed vegetable saps from the depression of the freezing point. *Amer. Jour. Bot.* 2: 418-19. 1915.
16. ———, LAWRENCE, J. V., and GORTNER, R. A. On the osmotic pressure of the juices of desert plants. *Science* 41: 656-58. 1915.
17. ———, LAWRENCE, J. V., and GORTNER, R. A. The cryoscopic constants of expressed vegetable saps as related to local environmental conditions in the Arizona deserts. *Physiol. Res.* 2: 1-49. 1916.
18. ———, and LAWRENCE, J. V. On the osmotic pressure of the tissue fluids of Jamaican Lorantheae parasitic on various hosts. *Amer. Jour. Bot.* 3: 438-55. 1916.
19. ———. Physical chemistry in the service of phytogeography. *Science* 46: 25-30. 1917.
20. ———, and LAWRENCE, J. V. Cryoscopic determinations on the tissue fluids of plants of Jamaican coastal deserts. *Bot. Gaz.* 64: 285-305. 1917.
21. ———, and LAWRENCE, J. V. The osmotic concentration of the tissue fluids of Jamaican montane forest vegetation. *Amer. Jour. Bot.* 4: 268-98. 1917.
22. ———, GORTNER, R. A., and LAWRENCE, J. V. The relationship between the osmotic concentration of leaf sap and the height of leaf insertion in trees. *Bul. Torrey Bot. Club* 44: 267-86. 1917.
23. ———. On the osmotic concentration of the tissue fluids of desert Lorantheae. *Mem. Torrey Bot. Club* 17: 307-15. 1917.
24. ———, and LAWRENCE, J. V. The osmotic concentration of the sap of the leaves of mangrove trees. *Biol. Bul.* 32: 202-11. 1917.
25. ———. On the osmotic concentration of the tissue fluids of Phanerogamic epiphytes. *Amer. Jour. Bot.* 5: 490-506. 1918.
26. ———, GORTNER, R. A., and LAWRENCE, J. V. On the relationship between the freezing point lowering and the specific electrical conductivity of plant tissue fluids. *Science* 52: 494-95. 1920.

27. ———, GORTNER, R. A., and LAWRENCE, J. V. On the differentiation of the leaf tissue fluids of ligneous and herbaceous plants with respect to osmotic concentration and electrical conductivity. *Jour. Gen. Physiol.* 3: 343-45. 1921.
28. ———, GORTNER, R. A., and LAWRENCE, J. V. The osmotic concentration and electrical conductivity of the tissue fluids of ligneous and herbaceous plants. *Jour. Phys. Chem.* 25: 122-46. 1921.
29. ———, and others. Maximum values of osmotic concentration in plant tissue fluids. *Proc. Soc. Exp. Biol. and Med.* 18: 106-109. 1921.
30. ———, and others. The osmotic concentration, specific electrical conductivity, and chlorid content of the tissue fluids of the indicator plants of Tooele Valley, Utah. *Jour. Agr. Res.* 27: 893-924. 1924.
31. ———. A table to facilitate correction for undercooling in cryoscopic work. *Amer. Jour. Bot.* 12: 499-501. 1925.
32. HARVEY, R. B. Hardening process in plants and developments from frost injury. *Jour. Agr. Res.* 15: 83-112. 1918.
33. KNUDSON, L., and GINSBERG, S. Suggestions with respect to the measurement of osmotic pressure. *Amer. Jour. Bot.* 8: 164-70. 1921.
34. KORSTIAN, C. F. Density of cell sap in relation to environmental conditions in the Wasatch Mountains of Utah. *Jour. Agr. Res.* 28: 845-907. 1924.
35. LEWIS, G. N. The osmotic pressure of concentrated solutions and the laws of the perfect solution. *Jour. Amer. Chem. Soc.* 30: 668-83. 1908.
36. LIDFORSS, B. *Die Wintergruene Flora*. Lunds. Univ. Arsskr. 2. 76 p. (Cited by Blackman (6), Chandler (7), Harvey (32), Maximow (40) and Newton (45).)
37. LIVINGSTON, B. E. The resistance offered by leaves to transpirational water loss. *Plant World* 16: 1-35. 1913.
38. ———, and SHREVE, F. The distribution of vegetation in the United States as related to climatic conditions. *Carnegie Inst. of Wash. Pub.* 284. 1921.
39. MARIE, C. H., and GATIN, C. L. Determinations cryoscopiques effectuées sur des sucres végétaux. Comparaison d'espèces de montagne avec les memes espèces de plaine. *Ass. franc. avanc. sci. (Dyon)* 40: 492-94. 1912.
40. MAXIMOW, N. A. *Chemische Schutzmittel der Pflanzen gegen Erfrieren*. *Ber. Deutsch. Bot. Gesell.* 30: 58-65, 293-305, 504-16. 1912.
41. ———. The physiological basis of drought-resistance of plants. (English abstract). *Leningrad* 1926.
42. MOLISCH, H. *Das Erfrieren von Pflanzen bei Temperaturen ueber dem Eispunkt*. *Sitzb. K. Akad. Wiss. (Vienna)* 105: p. 82, 95. 1896. (Cited by Harvey (32)).
43. MÜLLER-THURGAU, H. Ueber das Gefrieren und Erifrieren der Pflanzen. *Landw. Jahr.* 9: 133-89. 1880.
44. NEWTON, R., and GORTNER, R. A. A method for estimating hydrophilic colloid content of expressed plant tissue fluids. *Bot. Gaz.* 74: 442-46. 1922.
45. ———. The nature and practical measurement of frost resistance in winter wheat. *Univ. Alberta Res. Bul.* 1. 1924.
46. ———, BROWN, W. R., and MARTIN, W. M. The extraction of plant tissue fluids and their utility in physiological studies. *Plant Physiol.* 1: 57-65. 1926.
47. POOL, R. J. Xerophytism and comparative leaf anatomy in relation to transpiring power. *Bot. Gaz.* 76: 221-40. 1923.
48. ROSA, T. J., JR. The nature of hardening in vegetable plants. *Proc. Amer. Soc. Hort. Sci.* 16: 190-97. 1920.
49. SAYRE, J. D. The relation of hairy leaf coverings to the resistance of leaves to transpiration. *Ohio Jour. Sci.* 20: 55-86. 1920.
50. SCATCHARD, G. The hydration of sucrose in water solution as calculated from vapor pressure measurements. *Jour. Amer. Chem. Soc.* 43: 2406-18. 1921.
51. SCHAFFNIT, E. Ueber den Einfluss niederer Temperaturen auf die Pflanzliche Zelle. *Mitt. Kaiser Wilhelm Inst. Landw. Bromberg.* 3: 93-115. 1910.
52. TRANSEAU, E. N. Forest centers of eastern North America. *Amer. Nat.* 39: 875-89. 1905.
53. WARMING, E. *Lehrbuch der Oekologischen Pflanzengeographie*. 442 pp. Berlin 1902.
54. WIEGAND, K. M. Ice in plant tissue. *Plant World* 9: 25-39. 1906.
55. ———. The passage of water from the cell during freezing. *Plant World* 9: 107-118. 1906.
56. ———. Polarimetry. Circular 44, U. S. Bureau of Standards. Washington 1918.

THE ROCK SECTION AT THE O'SHAUGHNESSY DAM.

J. ERNEST CARMAN,
Department of Geology, Ohio State University.

For the purpose of securing additional water supply, the city of Columbus constructed during 1922 to 1925, a new dam on the Scioto river in the southwest corner of Delaware County about 15 miles northwest of Columbus. The excavations incident to the building of this dam exposed a section of rocks 90 feet thick which was studied from time to time by the writer.

The bed rock exposed along this portion of the Scioto valley is the Columbus limestone of middle Devonian age and the section that was exposed at the dam included the entire thickness of the Columbus and a few feet of the Delaware limestone above and of the Monroe dolomite below. The rocks exposed were correlated by zones with the standard section of the Columbus limestone for central Ohio as worked out by Dr. Clinton R. Stauffer and published in Bulletin 10 of the Geological Survey of Ohio.* The section as seen in the excavations during the building of the dam is given below:

	Thickness Feet
<i>Delaware Limestone—</i>	
Bluish limestone in layers of 3 to 6 inches.....	3+
<i>Columbus Limestone, 87 feet—</i>	
Zone H. Bluish gray, fossiliferous limestone in layers of 1 to 2 feet. The base is at a very smooth plane.....	7
Zone G. Bluish gray, fossiliferous limestone in layers of 3 to 5 feet....	19
Zone F. Bluish gray, very fossiliferous limestone with <i>Spirifer gregarius</i> (<i>Spirifer gregarius</i> zone).....	4
Zone E. Bluish gray limestone that weathers buff. In layers of 12 to 18 inches. Upper half fossiliferous.....	14
Zone D. Grayish brown limestone with bands of chert nodules and some chert layers (Chert zone). Contains a number of gastropods..	12½
Zone C. Missing.	
Zone B. Brown dolomitic limestone with some banding and in thick ledges of 3 to 5 feet. The basal 8 feet is more compact and contains some sand grains.	30
Zone A. Arenaceous limestone matrix with pebbles of Monroe dolomite and chert. (Conglomerate zone).....	½
<i>Monroe Dolomite—</i>	
Ashen gray to drab, laminated argillaceous dolomite in beds of 2 to 8 inches. In part brecciated, with minute fragments.....	2+

* 1909; Geol. Surv. Ohio, Bull. 10, pp. 32-38.

In Figure 1 is shown a cross section of the Scioto valley at the O'Shaughnessy dam as one sees it looking up stream from the south toward the face of the dam. The section, which is drawn to scale, shows the altitude and width of the various rock benches just south of the dam and the location of the exposures of the several zones of the Columbus limestone with respect to these benches and the valley sides. The zonal contacts are 2 to 3 feet lower on the east side than on the west, due to the general eastward dip of the rocks of the region. The elevation of the bed rock in the bed of the river here was at about 775 feet above sea level and the rock formed the valley sides up to about 845 feet.

The excavation for the foundation of the dam, reached the Monroe dolomite at 13 feet beneath the rock floor of the valley at 763 feet A. T., and extended 2 feet into the Monroe. The nearest exposure of this formation is 7 miles north-northwest on Mill creek near Bellpoint. North of White Sulphur, Delaware County, the Scioto river flows on the Monroe, but the southward dip of the rocks of this region, being greater than the gradient of the river, carries this formation below the bed of the river. The rock which was exposed in the bottom of the excavation at the dam is quite characteristic of the Monroe farther north in Delaware County. No Monroe fossils were seen but this is not surprising since many much larger exposures of the Monroe do not show fossils. It belongs to the Lower Monroe, or Bass Island formation, of Silurian age.

Zone A of the Columbus limestone is represented by a layer of conglomerate about 6 inches thick. The pebbles are laminated dolomite and chert from the Monroe. The matrix contains many well-rounded sand grains and in fact the sand content of the layer is greater here than at any other known exposure of this zone in central Ohio. The conglomerate layer rests disconformably upon the Monroe and the sides of the trench showed a contact irregularity of a few inches cutting across the layers of the Monroe. The zone represents the earliest deposit of the Columbus time as the sea transgressed the eroded, pebble-strewn surface of the Monroe. The sand grains were either on the surface or washed in by the encroaching sea. Their characteristics are such as to show that they were derived from the Sylvania sandstone of northern Ohio, the horizon of which is at this Monroe-Columbus hiatus.

Zone B is present on the three lower benches on the west and on the lowest bench on the east. It is a brown, dolomitic limestone in thick ledges of 3 to 5 feet, and contains few fossils. Only the upper half is exposed now but in the excavations beneath the river bed, the entire zone with a thickness of 30 feet was shown. The basal 8 to 10 feet are more compact, bluer in color, and contain some sand grains throughout the limestone. This basal part passes into the typical brown limestone above and is considered simply a phase of the basal part of the zone, laid down while the pre-Columbus surface with its sand was not yet deeply buried.

Zone C of the standard section, characterized by an abundance of corals was not differentiated in this section although the exposure of the horizon was complete. At one place in the upper part of zone B, a chert lense yielded some small corals but it could hardly be called a coral zone.

Zone D, the chert or gastropod zone, is well represented by grayish brown limestone with bands of chert nodules and some chert layers. The greatest percent of chert and the largest number of gastropods were found at a few feet below the top. The limits of the zone are not very definite but it is most distinct on the west where it has a thickness of $12\frac{1}{2}$ feet which is greater than for any of the sections given by Stauffer. This thickness is sufficient to represent the combined thickness of zones D and C but there are no corals in the lower part of the zone.

Zone E with a thickness of 14 feet underlies on the east the three benches at elevations of 817, 812 and 807 feet and the 811 foot bench on the west. It is bluish gray limestone in thick layers and the upper half is quite fossiliferous with many specimens of the gastropods *Pleuronotus* and *Euryzone*.

The very fossiliferous limestone forming zone F underlies the 822 foot bench on the east side of the valley. It contains the characteristic fossil *Spirifer gregarius* which on some bedding planes is quite abundant. Aside from the presence of this fossil the 4 feet of limestone forming this zone might very well be included with Zone E.

Zone G underlies the highest bench on either side and is present in the outer walls of the valley. It is in thick ledges which break up to thinner beds on weathering. The top of the zone is marked by a smooth plane and this may be found at about 4 feet below the top of the rock exposure in the east wall

just south of the dam. This zone, as well as the other thick bedded zones, E and B, is broken by vertical and inclined joints.

Zone H forms the upper few feet of the rock wall on either side of the valley but the top of the zone is not reached.

Bluish limestone interpreted as Delaware limestone was taken from the bottom of a trench beneath the present roadway about 50 yards east of the east end of the dam. The elevation of this stone is only a few feet above the top of the exposure of Columbus limestone in the east bluff at the end of the dam but the contact of the two formations was not seen here. The bluish limestone that was exposed in this trench is like the basal Delaware as found in the quarries at the town of Delaware to the north and very different from the shaly basal zone of the Delaware as found along the Scioto river 10 miles south of the dam. On the west side of the valley about 100 yards northwest of the west end of the dam, in the head of a gully, 2 or 3 feet of the Delaware limestone are exposed above a stratum containing a few fish teeth, the "bone bed," which marks the top of the Columbus limestone. The Delaware stone here is similar to that taken from the trench east of the valley. Fragments of the "bone bed" were also taken from a trench beneath the present roadway less than 50 yards from the west end of the dam, the elevation of the contact here being at about 851 feet.

The data given above shows that the Delaware limestone which underlies the upland comes to within 50 yards of either end of the dam and the width of the Columbus limestone belt along the Scioto valley is here only one-fourth of a mile instead of one and one-half miles as shown on the maps of the Columbus quadrangle.* The elevation of the Columbus-Delaware contact is at about 850 feet, about 45 feet lower than on the maps noted above. This contact was also found in the large ravine from the east about one-half mile south of the dam and in the large ravine from the east about one-half mile north of the dam and in both places its elevation agrees with that found at the dam.

No attempt was made to make a complete collection of the fossils of the various zones of the Columbus limestone exposed at the dam. Such fossils as were collected are listed below and their distribution by zones shown.

* Geol. Surv. Ohio, Bull. 14, and U. S. Geol. Surv., Folio No. 197.

	ZONES					
	B	D	E	F	G	H
ANTHOZOA—						
<i>Aulacophyllum convergens</i> Hall.....			x			
<i>Cystiphyllum ohioensis</i> Nicholson.....						x
<i>Cystiphyllum vesiculosum</i> Goldfuss.....				x	x	
<i>Eridophyllum verneuianum</i> (Edwards and Haime).....			x	x		
<i>Favosites emmonsii</i> Rominger.....			x			
<i>Favosites turbinatus</i> Billings.....			x	x	x	x
<i>Heliophyllum cornicula</i> Edwards and Haime.....				x	x	
<i>Zaphrentis gigantea</i> Rafinesque.....		x	x	x		x
<i>Zaphrentis prolifica</i> Billings.....			*	x	x	x
HYDROZOA—						
<i>Stromatopora ponderosa</i> Nicholson.....	x		x			
BRACHIOPODA—						
<i>Atrypa reticularis</i> (Linnaeus).....	x	x	x		x	x
<i>Atrypa spinosa</i> Hall.....			x	x	x	x
<i>Camarotoechia billingsi</i> Hall.....	x					
<i>Chonetes mucronatus</i> Hall.....				x	x	x
<i>Meristella nasuta</i> (Conrad).....		x				
<i>Productella spinulicosta</i> Hall.....				x		
<i>Rhipidomella vanuxemi</i> Hall.....			x			
<i>Schizophoria propinqua</i> Hall.....			x		x	x
<i>Spirifer acuminatus</i> (Conrad).....				x		x
<i>Spirifer duodenarius</i> (Hall).....					x	
<i>Spirifer gregarius</i> Clapp.....			x	x	x	
<i>Spirifer grieri</i> Hall.....		x		x		
<i>Spirifer macrothyris</i> Hall.....			x			
<i>Spirifer manni</i> Hall.....			x			
<i>Spirifer varicosus</i> Hall.....		x				
<i>Stropheodonta hemispherica</i> Hall.....	x	x	x	x	x	x
<i>Stropheodonta perplana</i> (Conrad).....		x			x	
<i>Strophonella ampla</i> Hall.....			x			
PELECYPODA—						
<i>Conocardium cuneus</i> (Conrad).....	x	x	x	x	x	
<i>Modiomorpha subalata</i> (Conrad).....			*			
<i>Modiomorpha concentrica</i> (Conrad).....		x	x	x		
<i>Paracyclas elliptica</i> Hall.....					x	
<i>Pterinea flabellum</i> (Conrad).....			x			
<i>Sanguinolites sanduskiensis</i> Meek.....			x			
GASTROPODA—						
<i>Bellerophon pelops</i> Hall.....	x	x	x			
<i>Callonema bellatulum</i> Hall.....		x				
<i>Callonema lichas</i> (Hall).....			x	x	x	
<i>Callonema humile</i> Meek.....		x				
<i>Dentalium martini</i> (Whitfield).....		x		x		
<i>Pleuronotus decewi</i> Billings.....		*	x	x	x	
<i>Euryzone lucina</i> Hall.....			x	x		
<i>Hormotoma desiderata</i> Hall.....		x				
<i>Loxonema pexatum</i> Hall.....		x				
<i>Palaeotrochus kerneyi</i> (Hall).....			x			
CEPHALOPODA—						
<i>Orthoceras ohioensis</i> Hall.....		x	x			
<i>Poterioceras eximium</i> Hall.....					x	
<i>Ryticeras columbiense</i> Whitfield.....			x	x		
<i>Spyroceras thoas</i> Hall.....		x				
CRUSTACEA—						
<i>Coronura diurus</i> (Green).....			x	x		
<i>Phacops cristata</i> Hall.....			x			
<i>Proetus rowii</i> Green.....			x	x		

* Collected by J. E. Schaefer.

A SECOND RECESSIVE FACTOR FOR BROWN PERICARP IN MAIZE.*

MARION T. MEYERS.†

Seed of a strain of maize with a brown pericarp was obtained from eastern Ohio in 1922. Records of the existence of such corn in the Corn Belt go back some 50 or 75 years (Klippart 1860), it usually being referred to as "prehistoric" or by some term indicating that it was unusual. Seed having a similar brown pericarp was obtained in 1925 from a different variety of corn being grown in western Ohio. The determining factor for brown pericarp in this strain proved to be identical genetically with that in the strain obtained earlier from eastern Ohio.

PREVIOUSLY REPORTED TYPES OF BROWN PERICARP.

The genetics of two other types of brown pericarp in maize have been reported previously. The first and more complete study was made of the interaction of the *A a* factor pair either with the factor *P* or with the factors r^{ch} and *Pl*, as reported by Emerson (1921) and Anderson and Emerson (1923). The pericarp is pigmented when either the factor *P*, or the factors r^{ch} and *Pl* are present. With *A* and either *P*, or r^{ch} *Pl*, the pericarp is red or cherry, whereas with the homozygous recessive allelomorph *a*, the pericarp is brown or brownish. The pericarp pigment due to the *P* factor is insoluble in water, whereas the pigment developed due to the r^{ch} *Pl* factors is soluble in water, is an anthocyanin, and is similar to the anthocyanins conditioned in other parts of the plants by a system of interacting factors of which the pairs *A a*, *R r*, and *Pl pl*, are members.

Anderson (1925) has reported a factor for brown pericarp which is dominant to red, in corn from Ecuador. Unlike the *A a* factor pair which affects all pigments except green and yellow in all plant parts, this factor affects pigments in the pericarp only, so far as known.

* The investigations reported in this paper were carried on at the Ohio State University in cooperation with the Ohio Agricultural Experiment Station and the United States Department of Agriculture.

† Instructor in Farm Crops, The Ohio State University; Assistant Agronomist, The Ohio Agricultural Experiment Station; Agent, United States Department of Agriculture.

GENETIC RELATIONS OF THE NEW BROWN PERICARP FACTOR.

The brown pericarp corn found in Ohio has been found to be distinct genetically from those previously reported, as shown in the following data from crossing experiments. The factor pair responsible accordingly has been designated *Bp bp*.

Interaction with the P p factor pair.

Several crosses were made between material homozygous for the new brown pericarp from Ohio and ordinary clear pericarp strains. All of the F_1 ears of this cross were red. The combined results from all the F_2 populations from such crosses are given in Table 1.

TABLE I.

NUMBERS OF EARS WITH RED, BROWN, AND CLEAR PERICARP IN F_2 PROGENIES FROM THE CROSS, BROWN $\times$ CLEAR PERICARP, (*P bp* $\times$ *p Bp*).

	Red Pericarp	Brown Pericarp	Clear Pericarp
Observed frequencies..	199	67	88
Calculated frequencies on basis of 9:3:4 ratio.	199.12 $\pm$ 6.30	66.38 $\pm$ 4.95	88.50 $\pm$ 5.50
Deviation.....	.12	.62	.50

This clearly is a modified dihybrid ratio without linkage. The effect of the homozygous recessive factor for brown pericarp *bp bp* is to modify the pigment whose presence is determined by the *P* factor, from red to brown. Combining the red and brown classes in Table 1, there were 266 pigmented to 88 clear pericarp ears, obviously a 3 : 1 ratio. No pigment develops in the presence of homozygous recessive *p*, regardless of the condition of the *Bp bp* factor pair, hence the 3 : 1 ratio between the pigmented and non-pigmented ears.

The *P p* factors are members of an extensive series of multiple allelomorphs controlling the distribution and intensity of a water insoluble pigment in the pericarp and cob, (Emerson 1917, Hayes 1917, Anderson 1924 and Eyster 1925). One of this series determines variegation in pigmentation. A variegated brown ear was obtained from a selfed plant grown from an

open-pollinated brown seed. The plants grown from the seed of this ear produced mostly brown variegated ears and a few self-brown ears. A selfed plant from a related open-pollinated brown seed produced a red ear. The F_2 progeny from this ear consisted of 32 self-colored red, 10 variegated-red, and 9 self-colored brown ears, and 1 variegated-brown ear. Evidently the action of the $Bp\ bp$ factor pair is the same with at least one additional member of this extensive $P\ p$ series, modifying only the color and not the distribution of the pigment.

Relation to the $A\ a$ factor pair.

The ears of this new brown type were indistinguishable phenotypically from the brown ears resulting from the interaction of homozygous recessive a with the P or $r^{ch}\ Pl$ as noted

TABLE II.

NUMBERS OF EARS WITH RED, BROWN, AND CLEAR PERICARP, PRODUCED IN F_2 PROGENIES FROM CROSSES BETWEEN HOMOZYGOUS BROWN PERICARP AND RECESSIVE $a\ a$ PLANTS ($P\ A\ bp \times p\ a\ Bp$).

	Red Pericarp	Brown Pericarp	Clear Pericarp
Observed frequency...	365	286	230
Calculated frequency on basis of 27:21:16 ratio.....	371.67 $\pm$ 9.89	289.08 $\pm$ 9.40	220.25 $\pm$ 8.67
Deviation.....	6.67	3.08	9.75

above. The $A\ a$ factor pair, however, influences pigments in other parts of the plant than the pericarp. When the production of brown is the result of segregation of the $A\ a$ factor pair, seeds with red or blue aleurone, and plants that are purple, dilute purple, sun red or dilute sun red always produce ears with either red or clear pericarp, but never ears with brown pericarp. A cross between two plants homozygous for recessive a would result in seeds with colorless aleurone and in either brown or green plants in the F_1 generation. Plants of the new brown pericarp type were crossed repeatedly with a -tester and brown plants grown from seed obtained from Dr. R. A. Emerson of Cornell University. In every case the F_1 endosperm had colored aleurone or the F_1 plants were purple and the pericarp red,

establishing the fact that a factor other than the *A a* allelomorphs was responsible for the brown in the new material. Moreover, brown pericarp ears occurred on F_2 plants carrying anthocyanin pigment as well as on brown and on green plants. The F_2 populations from such tri-hybrid crosses produced ears with red, brown and clear pericarp, as shown in Table 2.

The 27 : 21 : 16 ratio is expected if the three factors *A*, *P*, and *Bp* are not linked, if the *A a* and the *Bp bp* factor pairs are complementary in their interaction with the *P* factor, and if ears of the constitution *P a bp* are brown. The fit between the observed and the calculated frequencies for this ratio is very good, the highest value for $\frac{\text{Dev.}}{\text{P. E.}}$ being only 1.12.

The plants homozygous for both *a* and *bp* in the F_2 populations from this cross constitute a new combination. The recessive condition of either factor alone is sufficient to condition brown pigment in the presence of dominant *P*. If different steps in the physiology of the development of the pigment were disrupted by the two factors it is conceivable that the whole process might fail to come to visible completion or might give an end result different from either red or brown in the double recessive. If the ears from *P a bp* plants had clear pericarps the expected ratio in Table 2 would be 27 red; 18 brown; 19 clear, with a calculated frequency of 371.67 ± 9.89 red; 247.78 ± 9.00 brown; 261.54 ± 9.15 clear. This gives a poorer fit than before, the deviations of the calculated from the observed frequencies being increased from 0.33 times to 4.25 times the P. E. in the brown class, and from 1.12 times to 3.45 times the P. E. in the clear class, the two classes affected by this change. Apparently the effect of the combination of the two factors in the homozygous recessive condition is not different from the effect of either alone.

Linkage with the Wx wx factor pair.

Eight linkage groups have been recognized up to the present time in corn. The factor pair for waxy endosperm *Wx wx* is in Group I. Crosses were made between plants homozygous for brown pericarp and *wx wx* plants. The F_1 plants from this cross were backcrossed to *bp bp* plants. The plants segregating for the waxy factor in the resulting population were determined by testing the pollen of each plant with chloral-hydrate iodine.

The polysaccharide reserve of pollen grains bearing the *wx* factor stains red with iodine while the polysaccharide reserve of pollen grains bearing the *Wx* factor stains blue, (Demerec 1924, and Brink and MacGillivray 1924). The ears then were classified at harvest to obtain data on the segregation for the factor for brown pericarp. The distribution obtained in the classification of these backcross populations is given in Table 3.

TABLE III.

NUMBERS OF EARS WITH RED AND WITH BROWN PERICARP ON *Wx wx* AND *Wx* PLANTS

FROM THE BACKCROSS	$\frac{P \ Wx \ bp}{p \ wx \ Bp} \times P \ Wx \ bp.$	
	EARS WITH	
	Red Pericarp	Brown Pericarp
Plants segregating for <i>Wx wx</i>	49	9
Plants homozygous for <i>Wx</i>	9	56

The 18 cross-overs in a total of 123 plants indicate a crossing-over percentage of 14.63 ± 3.19 per cent.

SUMMARY.

The genetic relationships of a second recessive factor for brown pericarp in maize are reported.

The new factor, designated *bp*, is recessive to its normal allelomorph *Bp* for red, and is expressed only in interaction with the dominant *P* factor for pericarp color.

The factor *bp* is distinct from the *A a* factor pair and, although complementary with the *A a* factor pair in its behavior in interaction with large *P* factor, there is no interaction between *Bp bp* and *A a*.

The *Bp bp* factor pair is linked with the factor pair for waxy, *Wx wx*, with 14.63 ± 3.19 per cent crossing over, as determined from a back-cross population of 123 plants.

LITERATURE CITED.

- ANDERSON, E. G. 1924. Pericarp studies in maize II. The allelomorphism of a series of factors for pericarp colors. *Genetics* 9: 442-453.
- , 1925. A dominant brown pericarp color in maize. *Mich. Acad. of Sci., Arts and Letters* 5: 73-75.
- ANDERSON, E. G. and EMERSON, R. A. 1923. Pericarp studies in maize I. The inheritance of pericarp colors. *Genetics* 8: 466-476.
- BRINK, R. A., and MACGILLAVRAY, J. H. 1924. Segregation for waxy character in maize pollen and differential development of the male gametophyte. *Amer. Jour. Bot.* 11: 465-469.
- DEMEREK, M. 1924. A case of pollen dimorphism in maize. *Amer. Jour. of Bot.* 11: 461-464.
- EMERSON, R. A. 1917. Genetic studies of variegated pericarp in maize. *Genetics* 2: 1-34.
- , 1921. The genetic relations of plant colors in maize. *Cornell Univ. Agr. Expt. Sta. Memoir* 39: 1-156.
- EYSTER, W. H. 1925. Mosaic pericarp in maize. *Genetics* 10: 179-196.
- HAYES, H. K. 1917. Inheritance of a mosaic pericarp pattern color of maize. *Genetics* 2: 261-281.
- KLIPPART, J. H. 1860. *The Wheat Plant*. p. 672. Moore, Wilstach, Keys and Co., Cincinnati.

THE PRIMARY PLANT ASSOCIATIONS OF OHIO
THEIR DISTRIBUTION AND THEIR SIGNIFICANCE AS
HABITAT INDICES.*†

HOMER C. SAMPSON,
Department of Botany, Ohio State University.

INTRODUCTION.

The idea of natural plant associations as indices of habitat differences is a very old one. Pioneers everywhere have used it. There is now no adequate record of these primeval plant associations, and they have been so obscured by the effects of civilization that if we wish to use them as natural indices to habitats, we must first reconstruct them from present remnants and the secondary associations that follow clearing, grazing, and other destructive processes of man.

The reconstruction of the vegetation of the past from present day conditions is often a difficult task, and it is frequently neglected in field surveys. As evidence one may cite the relatively large numbers of papers intended as descriptions of natural plant associations in various parts of the country in which the authors fail to recognize secondary associations and actually describe them as original vegetation. The distinction between primary and secondary associations is one of the first problems that should be met and solved by anyone wishing either to describe the vegetation of a region, or to discover the relations of natural vegetation to soils, climatic factors, or other natural phenomena.

The map before you is the result of an attempt by Dr. E. N. Transeau and myself to determine the distribution of the natural vegetation of Ohio as it occurred about a century and a half ago, *i. e.*, before it had been disturbed except by Indian occupancy. The data obtained in the field surveys and the conclusions reached are being checked wherever possible by data available in the earlier reports on the vegetation of the state found in county histories, reports of travelers, early

* Paper read before the general session of the Ohio Academy of Science, April, 1927.

† Papers from the Department of Botany, The Ohio State University, No. 189.

geological surveys, and surveyors' records. Sears* has recently published a map of the "Ohio Virgin Forest" based upon data obtained from these early records. Some of the plant associations recognized in our field survey are indicated on this map. Their general distribution in the state as determined by Sears from early records agrees with the general distribution we have assigned them by field studies. This map is of value, therefore, as a check on the reliability of our field methods of reconstructing the original vegetation.

Several vacation periods were spent in studying the least disturbed areas, remnants of original forests, and numerous secondary forests in various parts of Ohio and neighboring states. In this preliminary work emphasis was placed on learning to recognize the several plant associations, both primary and secondary, and also the successions that might occur among them. We then began to map the distribution of the original plant associations of the entire state. Last summer Dr. L. L. Huber, Dr. J. S. Houser, and Director Williams of the Ohio Experiment Station saw that our data had a bearing on some of the problems in which they are interested and furnished us financial aid. If this assistance continues we shall have the vegetation map ready for publication much sooner than we had anticipated.

Geological and soil maps of the state have been published and attempts have been made to correlate the natural vegetation with these maps. It is mainly of these mutual interests that I wish to speak today: to point out (1) some of the features of the natural vegetation that should be considered in future attempts to determine such correlations as exist, and (2) that the distribution of the natural plant associations of the state is itself an index of environmental conditions with potentially distinct uses and value.

THE PRIMARY PLANT ASSOCIATIONS.

Previous to the nineteenth century there were more than thirty well recognized primary plant associations in the state. Four of these associations: Beech-Maple, Elm-Ash-Maple, Oak-Hickory, and Mixed Mesophytic are of first importance on the basis of area covered. Their distribution is indicated on the

*Sears, P. B. The natural vegetation of Ohio. Ohio Jour. Sci. 25:139-149, 1926.

map.* Of secondary importance on the basis of area covered by the association are: Willow, Alder, River Birch, and Maple-Cottonwood-Sycamore Associations along some of the streams; Oak-Chestnut or Oak-Chestnut-Hickory on sandy or gravelly areas, dryer talus and hill tops, and certain stages of cliff erosion; White Pine in northeastern Ohio and Pitch Pine in southeastern Ohio as cliff associations; Post Oak-Bur Oak between Oak-Hickory and prairie and as isolated groves on the prairie; Oak-Maple as a transition between Beech-Maple and Oak-Hickory near the prairie, but usually not elsewhere; Slough Grass and Andropogon associations of the wet and dry prairies respectively; Scirpus-Typha-Phragmites marshes; Cranberry, Alder, Tamarack, Aborvitae, White Pine and Hemlock associations of bogs; Birch, Hemlock-Birch, and Beech-Maple-Hemlock-Birch associations in gorges, deep ravines, and on steep slopes. The last named association also occurred on uplands in the extreme northeastern part of the state. Since the map is on display where you may observe the distribution of these associations at your convenience, further details of distribution will be omitted at this time.

The present distribution of these associations represents the latest moves in a long series of successions. The succession of plant associations is now a well established law in biology and should be recognized in all modern field studies. As the habitat factors change, the associations change, and these changes follow orderly sequences that may be detected by appropriate methods of investigation.

The successions that should be kept in mind in studying natural vegetation in this state are of three types. First, the historical successions of a geographical order including (a) successions following the retreat of the glacier, (b) successions during the post-glacial xerothermic period, (c) successions subsequent to that period. Second, the primary successions accompanying the changes in soil and atmosphere of the present time. Third, the numerous secondary successions following clearing and other activities of man. Several associations in the state are lingering relics of the first type of succession noted above.

* Since this is mainly a report of progress and suggestions for future investigations, the map in its present stage of development is omitted from publication.

SIGNIFICANCE OF THE PRIMARY PLANT ASSOCIATIONS AS HABITAT INDICES.

The first point of importance is that the primary plant associations represent a summation of both the atmospheric and soil factors acting over a period of years.

As a point in evidence it may be noted that their general distribution in the state is not coincident with geological formations, glaciation, soil types, temperature, rainfall, or any other single environmental factor or restricted group of environmental factors. Their distribution is a resultant of all the atmospheric and soil factors acting simultaneously.

The effects of certain environmental factors are of course more prominent than those of others. But the factors showing the most prominent effects in one locality of the state, are not necessarily the most prominent in effect in another locality. For example as we approach the prairie areas of Ohio Beech-Maple disappears, first from the uplands then from the ravines. Oak-Maple becomes the usual forest for a few miles, then Oak-Hickory becomes the common forest on all the uplands. Exactly the same changes in forests occur as we go westward and approach the prairie climate of the Central States. This similarity in the changes of forests in the two cases indicates that atmospheric factors are most effective in determining the Oak-Hickory association in certain parts of the state. On the other hand, topography and soil factors becoming unfavorable for Beech-Maple are as clearly the most effective factors accounting for Oak-Hickory in certain other localities.

As another example we may choose a single soil type*, say Wooster loam, and if a rather extensive area of this soil type is traversed we can find growing upon it associations of Oak-Chestnut, Oak-Hickory, Beech-Maple, Oak-Maple, and Mixed Mesophytic. This dissimilarity of plant associations on the same soil type results mainly in some instances from differences in atmospheric factors, in other instances from changes in the soil factors most effective in plant growth but not given the most weight in classifying the soil type. Furthermore these same five associations occur on other soil types in other parts of the state. Or the same association, such as Beech-Maple, occurs on numerous soil types. This similarity of plant associations on different soil types shows that some of the soil character-

* The distribution of soil types was obtained from published maps.

istics chosen to distinguish soil types may hold a very minor position among the factors that affect plant development and distribution. Where soil types are distinguished on the basis of variations in water and oxygen content, interesting relations of soil type and vegetation do occur locally.

Another condition that should be recognized is that the relative effects of soil and atmospheric factors varies geographically. Transeau emphasized this point several years ago in his paper on "Forest Centers."* He recognized in each of the climatic plant formations of the continent a "center of distribution" in which the plants of that formation reached their best development. One of the characteristics of this center of distribution is that the differential effects of topography and other soil factors are less effective there than elsewhere in the formation, and that as one departs farther and farther from the center of distribution the relative effects of topography and soil become more noticeable.

It must also be remembered that the effect of one plant association upon another, the biotic effect, is a factor in their distribution. So far as climate and soil alone are concerned the Oak-Hickory association would occupy most of the area of the state. But when these conditions become suitable also for Beech-Maple or Mixed Mesophytic forests, Oak-Hickory is shaded out. However if clearing continues, secondary Oak-Hickory will occupy most of the area originally covered by these associations. The chief exceptions are the secondary forests of swamp forest species following the clearing of Beech-Maple on poorly drained areas.

A second point of importance is that if we may add to our knowledge of the primary associations an understanding of the secondary associations that follow the first clearing of the primary, we shall have a still better basis for the evaluation of the atmospheric and soil factors.

The secondary forests following a single clearing of the primary associations are not the same in different parts of the state. As an example, the secondary forests that may follow a single clearing of the Beech-Maple association in various parts of the state are: Beech-Maple, Maple-Beech, Oak-Maple, Oak-Hickory, Oak-Chestnut, Mixed Mesophytic, and Elm-Ash-Maple.

*Transeau, E. N. Forest Centers of eastern North America. *Am. Nat.* 39:875-889, 1905.

An interpretation of these variations in secondary forests in relation to atmospheric and soil factors in a given region can frequently not be made without a thorough knowledge of the biotic factors involved, both present and historical. A knowledge of these secondary associations following the first, second, and third clearings would have its uses, particularly in the field of forestry. Clearing is making an enormous change in the proportion of the state occupied by each of the several forest associations.

The third point of importance is that since the natural plant associations represent a summation of the atmospheric and soil factors, they ought to serve as convenient indices of biological habitats in the study of certain agricultural problems. Along with other general indices of habitats, such as geological formations, soil types, and summations of atmospheric conditions, they should serve as an additional and a distinctly different type of measure of habitat conditions. No single index or unit of measure will suffice.

Is there any evidence that the natural plant associations may be used as indices to biological habitats in the state under present agricultural conditions? I have already indicated their possible relations to forestry problems. We are of the opinion that a knowledge of the primary and secondary associations and of the successions among them, will furnish a valuable foundation upon which to predict the types of forests that may most readily be maintained in different parts of the state. But the final working out of these correlations must be delegated to the forester.

The most carefully analyzed correlation to date is that between the distribution of natural plant associations and certain insect infestations in the state. In 1924 Mr. Merlin Jones a student in entomology at this University furnished us a map of the distribution and of the degree of infestation of the Mexican bean beetle in this state. When Jones' map was superimposed upon our vegetation map there was seen to be a close correlation between the distribution of the Mixed Mexophytic forest and the areas in which the Mexican bean beetle was doing commercial damage. Field studies last summer by Dr. Transeau and Mr. N. F. Howard of the U. S. Bureau of Entomology showed this correlation to be surprisingly constant.

When the corn borer invasion became alarming, Dr. Transeau suggested that it would probably not do commercial

damage in all habitats and that the natural plant associations might furnish an index to the habitats in which it would do commercial damage. Dr. L. L. Huber had already noted in 1924 that the degree of infestation varied locally with certain habitat conditions. The Experiment Station agreed that the idea was worth investigation.

The conditions in Ontario were used as a preliminary test case. We determined the distribution of the primary plant associations in the entire corn borer area of Ontario. When our vegetation map was compared with maps of the corn borer infestation furnished by the entomologists* it was found that the corn borer had been doing commercial damage in Ontario only in those areas originally occupied by the Swamp Formation, *i.e.*, the series of successions from marsh grasses through intermediate associations of bog and swamp vegetation to the Elm-Ash-Maple swamp forest association of the Eastern Deciduous Forest. The borer had been present equally long in other habitats in Ontario but its devastation there had not been considered of commercial significance. The survey was then extended to include the corn borer area in Ohio and neighboring states. At the close of the season our vegetation maps were compared with Dr. Huber's maps of the distribution and the degree of infestation of the corn borer. The correlation discovered in Ontario was found to hold throughout the entire region investigated.† These two examples of insect devastation indicate how the natural plant associations may serve as convenient indices in predicting the habitats in which the depredation of certain insects should either be controlled or avoided.

Perhaps the behavior of some of our cultivated plants will show equally interesting correlations. But students familiar with the behavior of cultivated plants must be persuaded to help supply and evaluate the data necessary to bring out such correlations as exist. Mr. J. H. Gourley of the Ohio Experiment Station suggested in conversation that most of the com-

*Numerous entomologists contributed data and personal assistance. General acknowledgment is made to Dr. L. L. Huber and Dr. C. R. Neiswander, of the Ohio Experiment Station; to H. G. Crawford, Chief of the Division of Field Crops and Garden Insects, Canada; and to Dr. E. P. Felt, State Entomologist, New York.

† A paper on "Vegetation Types and Insect Devastation," by E. N. Transeau, is to appear in the July number of *Ecology*, and may be consulted for a more detailed account of this topic.

mercial orchards in Ohio are in that part of the State where our map shows an abundance of the mixed mesophytic forest in the valleys and of Oak-Hickory on the hilltops. Orchard trees as perennial plants are subject to the environmental factors of the habitat during the entire year just as are the natural plant associations, while many other cultivated plants are annuals and subject to the factors of the habitat only during the growing season. It is beyond the scope of this paper to attempt an enumeration of the complexities of the situation. I wish merely to call attention to two more conditions that are in keeping with the general tenor of this discussion.

First, agricultural practices modify many of the factors of the natural habitat. The general effects of these modifications can be appreciated at once if we direct our attention to the manner in which external factors frequently affect plant development and distribution. The general conclusion that may be drawn from the data of numerous field studies and experimentation on the causes of the differences in distribution of the plant associations characteristic of this region is that the external factors foremost in limiting the distribution of these associations in Ohio are the factors that bring about within the plant either desiccation, starvation, or suffocation. The first condition is usually brought about by a deficiency in external moisture, at least during the growing season; the second usually by overshadowing by other plants; and the third by excess water leading to oxygen deficiency. The effects of other factors are less prominent, except perhaps for a few individual species. For example the species of the Oak-Hickory association can not withstand the desiccation of the most arid habitats in the state, their roots suffocate during the long submergence in swamps and floodplains, and they are unable to manufacture food as rapidly as they use it in the shade of beech and maple. Any agricultural practice that modifies either of the three sets of conditions named above may bring about modifications in expected correlations.

The second point is that any attempt to correlate the behavior of cultivated plants with the natural associations should not ignore the successional history of different localities. For example, two years ago I listed the successional series that have led to the Oak-Hickory and Elm-Ash-Maple associations in northeastern Ohio. The Oak-Hickory association was found to have succeeded such xerophytic associations as Pine and Oak-

Chestnut, prairie vegetation, and even swamp forest on a peculiar hydriphyllous clay soil. As a secondary association it had succeeded Beech-Maple and Mixed Mesophytic. The Elm-Ash-Maple swamp forest association had succeeded the swamp vegetation of marshes, pioneer floodplain forests, and relic bog vegetation. As a secondary association it had succeeded tamarack and other conifers on bogs, and Beech-Maple on poorly drained areas.

These different successional series of vegetation leave different effects in the soils upon which they occur and these differences may be reflected in the behavior of cultivated plants. In such cases a uniform correlation would not occur.

It is apparent that such correlations and lack of correlations as I have cited can be determined only by having each kind of survey made by specialists in the fields concerned. Our chief interest in this survey is to discover and interpret the behavior of natural plant associations. The vegetation map is of value to us as a basis in selecting critical stations for quantitative studies of the relative effects of environmental factors throughout the season. In the last analysis understanding and practice both require an evaluation of the external factors in their relation to the physiology and development of the different species and varieties. General indices to habitats have certain values, but they will not remove the need of studies of the relative effects of the separate factors of the habitat during critical periods of the year and at different stages in the development of the plant.

NEW BOOKS.

THE CENTURY COMPANY has recently issued two attractive books on scientific subjects which we are glad to bring to the attention of our readers. One of them, *Oxidation-Reduction Reactions In Inorganic Chemistry*, by Eric R. Jette, will be of interest to chemists, and the other, *Host Parasite Relations Between Man and His Intestinal Protozoa*, by Robert Hegner, will interest both the zoologist and the public health worker. The publisher's statements regarding these volumes are given below:

Host-Parasite Relations Between Man and His Intestinal Protozoa—By ROBERT HEGNER, PH. D., Professor of Protozoology in the School of Hygiene and Public Health of the Johns Hopkins University. Price, \$3.50.

The purpose of this book is to present the more relevant data regarding the host-parasite relations of the intestinal protozoa of man in such a manner as to show the present state of our knowledge and to focus attention on the need for more systematic and intensive research in the subject. The distinguishing feature of this study is its effort to co-ordinate the zoological phases of the subject with the medical phases and both the zoological and medical phases with methods of prevention and control. The control of the parasitic species, to be effective, must be based on a knowledge of the relations between the parasite and its host. What these relations are and the little we know about them is explained in this book.

This volume is the first to appear in The Century Biological Series, of which Dr. Hegner is the general editor. It is illustrated with charts, diagrams, and half-tone plates and contains a valuable twenty-five page list of references to the literature in the subject and an index of the authors quoted or referred to in the text.

Oxidation-Reduction Reactions In Inorganic Chemistry—By ERIC R. JETTE, PH. D., Assistant Professor of Chemistry at the Washington Square College of New York University. Price, \$1.10.

This book presents a comprehensive discussion of oxidation-reduction reactions for the student who has had enough training in chemistry to understand ordinary chemical terminology, but who has not had the benefit of a course in physical chemistry. It emphasizes the qualitative, rather than the quantitative, aspects of the subject; and presents both the valence change and the ion-electron methods of balancing equations in order to enable the student to balance the equations for the greatest possible number of oxidation-reduction reactions. The book aims to develop the fundamental basis of each of these methods and, to this end, introduces the modern concepts of atomic structure, the ideas of polar and non-polar compounds, and certain elementary principles of electrochemistry. The ion-electron method is discussed in detail with numerous applications which illustrate the effect of solubility, degree of ionization, concentration of ions, complex-ion formation, etc., on oxidation-reduction reactions taking place in aqueous solutions.

INDEX TO VOLUME XXVII.

	PAGE
A Check List of Carabidæ of Columbus, Ohio.....	155
Additions to the Catalog of Ohio Vascular Plants for 1926.....	95
ALEXANDER, W. H.....	157
Alimentary Canal of the Mexican Bean Beetle.....	127
Alimentary Tract of the Bumble Bee.....	219
Annual Meeting of the Ohio Academy of Science.....	157
A Phaenological Note on the Flora of Cold Spring Harbor, N. Y....	138
A Second Recessive Factor for Brown Pericarp in Maize.....	295
BAKER, R. C.....	175
Biology of the European Corn Borer and Two Closely Related Species.....	47
Blood Vessels, Celloidin Injection of.....	200
Branchippus vernalis, Spermatogenesis of.....	175
Bryales, Ohio Mosses.....	1
Bumblebee, Alimentary Tract.....	219
CARABIDÆ of Columbus, Ohio.....	155
CARMAN, J. ERNEST.....	289
Celloidin Injection of Blood Vessels.....	200
Cissia mitchelli Found in Ohio, with Notes on its Habits.....	203
Corn, Diseases of.....	232
DESCRIPTION of Fifteen New Species of Ceratocapsus.....	143
Development of the Male Gonopods and Life History Studies of a Millipede.....	25
Diseases of Corn.....	232
DRAKE, C. J.....	102
EDDY, C. O.....	187
Enicocephalidæ, New Species of.....	102
European Corn Borer.....	47
EVERLY, R. T.....	155
FLORA of Cold Spring Harbor.....	138
Fusarium moniliforme in Relation to Diseases of Corn.....	232
GREEN, S. A.....	138
GRIER, N. M.....	138
HARRIS, HALBERT M.....	102
HENDERSON, NELLIE F.....	1
Heredity, Sex-limited Characters and Allosome-linked.....	105
INJECTION OF BLOOD VESSELS.....	200
KNIGHT, HARRY H.....	143

	PAGE
Leaves and Leaf Saps, Physical Properties.....	200
Lepidoptera (<i>Cissia mitchelli</i>).....	203
Life History Studies of a Millipede.....	25
LIMBER, D. P.....	232
MAIZE, Recessive Factor for Pericarp.....	295
MAXFIELD, FRANCIS N.....	205
MEYER, BERNARD S.....	263
MEYERS, MARION T.....	295
Mexican Bean Beetle, Alimentary Canal.....	127
MILEY, HUGH H.....	25
Millipede, Development of.....	25
MOORE, ROBERT H.....	200
Mosses, Bryales of Ohio.....	1
NATIVE Persimmon in Nature.....	187
New Species of <i>Ceratocapsus</i>	143
New Species of <i>Enicocephalidæ</i>	102
OHIO Academy of Science.....	157
Ohio Mosses, Bryales.....	1
Ohio Vascular Plants for 1926.....	95
O'Shaughnessy Dam, Rock Section.....	289
PALLISTER, JOHN C.....	203
Phaenological Notes on Flora of Cold Spring Harbor.....	138
Physical Properties of Leaves and Leaf Saps.....	263
Plant Taxonomy.....	249
POOS, F. W.....	47
POTTS, SAMUEL F.....	105
Principles in Plant Taxonomy.....	249
REPORT of the 37th Annual Meeting of Ohio Academy of Science..	157
Rock Section at O'Shaughnessy Dam.....	289
ROSOF, J. A.....	175
SCHAFFNER, JOHN H.....	19, 95, 105, 249
SCOTT, ERNEST.....	200
Sex-limited Characters and Allosome-linked Heredity.....	105
Sex-limited Characters in Heterosporous Sporophytes.....	19
Studies on the Physical Properties of Leaves and Leaf Saps.....	263
SWINGLE, MILLARD C.....	219
TAXONOMY, Principles of Plant.....	249
The Alimentary Canal of the Mexican Bean Beetle.....	127
The Alimentary Tract of the Common Bumblebee.....	219
The Celloidin Injection of Blood Vessels.....	200
The Observer: An Instrument of Precision.....	205
The Place of the Native Persimmon in Nature.....	187
The Rock Section at the O'Shaughnessy Dam.....	289
Three New Species of <i>Enicocephalidæ</i>	102
VASCULAR Plants, of Ohio.....	19